American Journal of Medical Genetics 68:99–104 (1997)

FISH Characterization of Small Supernumerary Marker Chromosomes in Two Prader-Willi Patients

D. Bettio,[1]* N. Rizzi,[1] D. Giardino,[1] F. Gurrieri,[2] G. Silvestri,[3] G. Grugni,[3] and L. Larizza[1,4]

[1]*Laboratorio di Citogenetica, Centro Auxologico Italiano, Milan, Italy*
[2]*Istituto di Genetica Medica, Università Cattolica, Rome, Italy*
[3]*Divisione di Endocrinologia e Divisione di Auxologia, Ospedale S.Giuseppe, Centro Auxologico Italiano, Piancavallo, Italy*
[4]*Dipartimento di Biologia e Genetica per le Scienze Mediche, Università di Milano, Milan, Italy*

A small supernumerary chromosome was observed in two Prader-Willi syndrome (PWS) patients. The clinical diagnosis of PWS was confirmed by the ascertainment of the deletion of region 15q11-13 in one case and uniparental disomy (UPD) of the same region in the other. The markers were negative for dystamycinA/DAPI banding, did not contain NOR-positive satellites, and had an appearance consistent with a very small ring chromosome. Fluorescent in situ hybridization (FISH) analysis with the "all human centromere" probe indicated the presence of centromeric sequences in both markers. Chromosomal in situ suppression hybridization with chromosome specific libraries demonstrated that the small markers in the deleted and UPD patient originated from chromosome 15 and X, respectively. To the best of our knowledge these are the only PWS patients reported with a supernumerary marker chromosome other than inv dup(15) characterized by FISH. Am. J. Med. Genet. 68:99–104, 1997

KEY WORDS: supernumerary marker chromosomes; Prader-Willi syndrome; FISH analysis

INTRODUCTION

Marker chromosomes are often difficult to characterize with classical cytogenetic procedures and the FISH techniques provide a means of identifying their origin [Callen et al., 1992; Daniel et al., 1994; Blennow et al., 1995].

Approximately 81% of the supernumerary marker chromosomes (SMC's) are derived from the acrocentrics [Blennow et al., 1994], the most common being inv dup(15) [Robinson et al., 1993]. Rare cases of Prader-Willi syndrome (PWS) with inv dup(15) have been reported, but the relationship of this marker to PWS has not been elucidated in most patients studied [Mattei et al., 1984]. Recently two PWS and two Angelman syndrome (AS) patients with inv dup(15) were studied at the molecular level and found to have either UPD or 15q11-13 deletion. In all cases it was hypothesized that the marker chromosome did not contribute to the patient's phenotype [Robinson et al., 1993; Cheng et al., 1994; Spinner et al., 1995].

We report on the fluorescence in situ hybridization (FISH) characterization of additional SMC's observed in two PWS patients and discuss the karyotype-phenotype correlations.

CLINICAL REPORTS

The patients were referred for genetic studies following a clinical diagnosis of PWS, established according to the consensus diagnostic criteria [Holm et al., 1993].

Case 1

The patient is the first of three children from healthy non-consanguineous parents. At his birth the parents were 30 years old. The boy was born in the 38th week of gestation by caesarian section because of fetal distress. Birth weight was 2,650 g. The clinical diagnosis of PWS was made when he was 5 years old. At 8 years he was hospitalized for Henoch-Schönlein purpura, successfully treated by steroids. When he was 18, right orchiectomy was carried out because of ectopic location of the testis. The phenotype of PWS was recognizable except for hypopigmentation and a typical face (Fig. 1).

Contract grant sponsor: Ministero della Sanita'; Contract grant number: 5504/ICSS49.4RS92/699

*Correspondence to: Dr. Daniela Bettio, Laboratorio di Citogenetica, Centro Auxologico Italiano, Via Ariosto, 13 20145 Milano, Italia.

Received 27 November 1995; Accepted 1 March 1996

Fig. 1. Patient 1 at age 27.

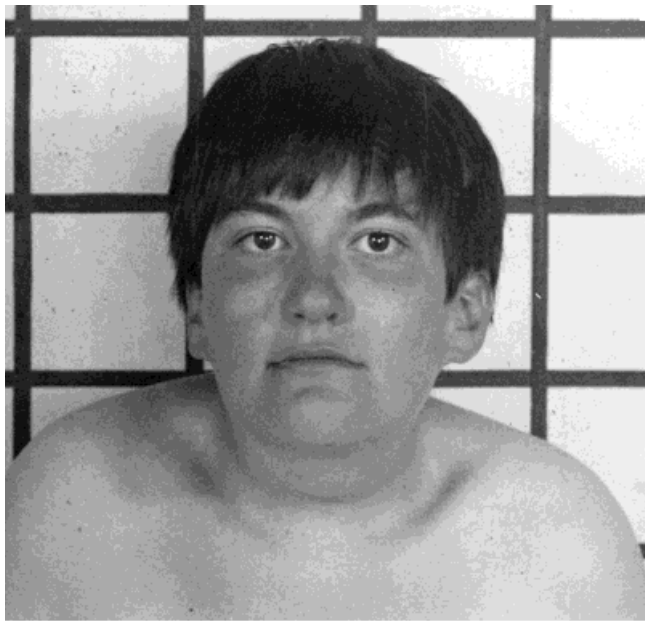

Fig. 2. Patient 2 at age 16.

Case 2

The proband is the fifth child of healthy, nonconsanguineous parents. The four sibs are healthy. The mother was 43 and the father 48 years old at the time of her birth. The girl was born at 40 weeks after an uneventful pregnancy. Birth weight was 3,650 g; delivery was normal. There was no history of seizures; nevertheless an electroencephalogram performed at 9 months showed signs of cortical-subcortical irritation, which disappeared at 5 years of age. She exhibited all the typical findings of PWS except for the characteristic face (Fig. 2).

CYTOGENETIC AND MOLECULAR STUDIES

Routine cytogenetic studies were performed on peripheral blood lymphocytes according to standard procedures. Banding techniques were QFQ, GTG, CBG, DA-DAPI, and NOR.

FISH analysis involved the use of D15S11, SNRPN, D15S10 and GABRB3 cosmids (ONCOR, Inc.) for the evaluation of the 15q11-13 deletion. Identification of SMC's was carried out with repetitive probes (all human centromere and D15Z1 classical satellite) and chromosome-specific libraries (ONCOR, Inc.). Three different chromosome paintings were mixed and hybridized simultaneously to the same chromosome spread. Specific chromosome libraries were used in sequence starting from chromosomes most commonly involved in SMC's according to the literature. When a positive hybridization signal was observed, each painting was used separately to identify the specific chromosome involved. In situ hybridization and immunochemical detection were carried out according to the protocol recommended by the supplier.

In the non-deleted PWS patient the methylation pattern study at the PW71 locus was performed as described by Dittrich et al. [1992].

RESULTS

Classical cytogenetic studies demonstrated the presence of an SMC in two PWS patients. Both markers appeared as very small ring chromosomes without any specific banding pattern, NOR and DA-DAPI negative (Fig. 3). FISH analysis, by means of the PWS/AS region specific probes, enabled us to identify the interstitial 15q11-13 deletion in patient 1. No PWS region-specific signal was apparent on the SMC, which was present in 70% of lymphocyte metaphases. The methylation pattern study showed the presence of the maternal band only in the non-deleted patient (case 2), who had the marker chromosome in 50% of the lymphocytes (Fig. 4). This finding confirms the PWS diagnosis in patient 2, indicating that she has either UPD or a defect of the imprinting center. This defect, reportedly rare, could not be ruled out by microsatellite analysis since both parents were deceased. This marker was FISH-negative with

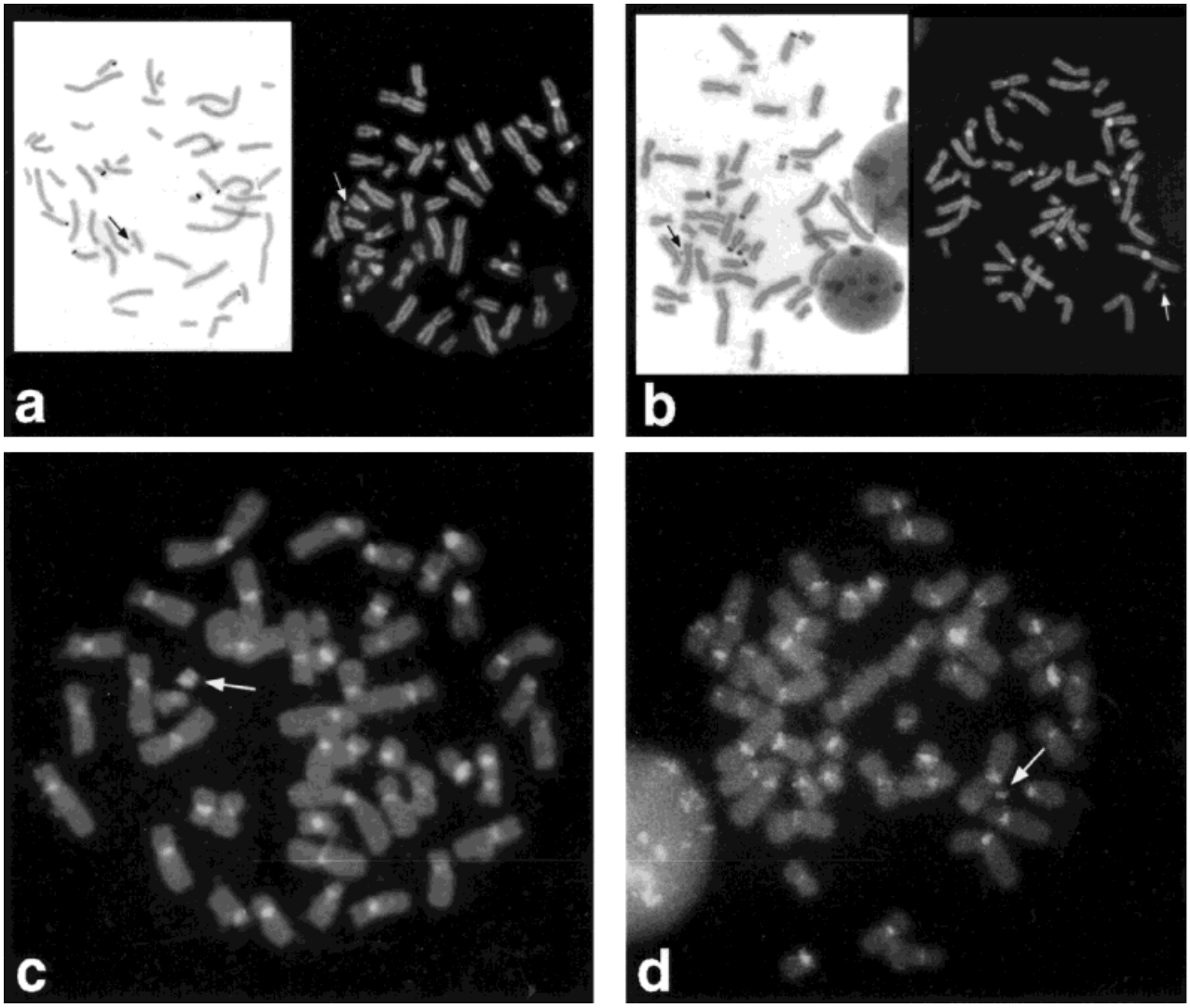

Fig. 3. Ag-NOR and DA-DAPI staining of metaphases from patient 1 (**a**) and patient 2 (**b**). Arrows indicate the negative markers. FISH with the "all human centromere" probe to metaphases from patient 1 (**c**) and patient 2 (**d**). A fluorescent signal (arrow) is visible on both markers.

the PWS region specific cosmids. Both SMC's had a centromeric region, as shown by the clear fluorescent signal given by FISH with the "all human centromere" probe (Fig. 3). In the deleted patient the marker showed no fluorescent spot after chromosome in situ suppression (CISS) hybridization with painting for chromosomes 4,6,7,8,9,14,18, and 20, but was positive to the combination 15+21+22. The use of each painting separately, established the chromosome 15 origin of the small ring. No hybridization signal was detected with the D15Z1 probe. In the case with UPD the marker was negative after CISS with the same chromosome libraries analyzed in case 1 plus those for chromosomes 12 and 13. The hybridization mix with libraries for chromosomes 3, 19 and X gave a positive signal, allowing the X chromosome origin of the marker to be established (Fig. 5).

DISCUSSION

FISH studies enabled us to demonstrate the chromosome 15 pericentromeric origin of an SMC in a PWS patient carrying a del 15q11-13 (case 1) and the X chromosome derivation of an SMC in a PWS patient with UPD (case 2).

Recently, several studies on marker chromosomes and their clinical significance have been reported [Daniel et al., 1994; Blennow et al., 1995; Brøndum-Nielsen et al., 1995]. In rare cases SMC's have been observed in PWS [Fujita et al., 1980; Wisniewski et al., 1980; Ledbetter et al., 1982; Mattei et al., 1984; Robinson et al., 1993] and AS patients [Robinson et al., 1993]. These markers, classified as inv dup(15), are generally small constituted by chromosome 15 short arm material and the most proximal sequences

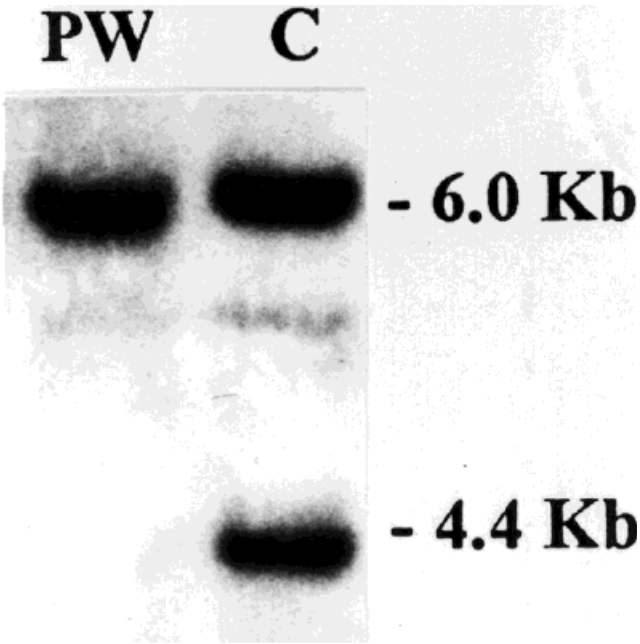

Fig. 4. Methylation analysis of patient 2 (PWS2) and control (c) DNA. Note the presence of the maternal allele (6.0 Kb) only in PWS2.

on chromosome 15q apparently not including the 15q11-13 region. This was confirmed by FISH in a deleted AS patient carrying an inv dup(15) [Spinner et al., 1995].

Although a higher frequency (25-fold) of this marker has been reported in PWS patients [1/40; Ledbetter et al., 1982] than in the general population [1/1,000; Sachs et al., 1987] we have not observed any inv dup(15) in a sample of 70 typical PWS patients we have analyzed so far. SMC's other than inv dup(15) have been reported in two PWS patients although their origin could not be established [Fleischer Michaelson et al., 1979].

In our patient 1, showing a 15q11-13 deletion, the marker was demonstrated to derive from the centromeric region of chromosome 15. The rare finding of a de novo del(15) and mar(15) in the same patient raises the possibility of a common cause in the origin of the two abnormalities. This hypothesis is supported by a few reports on patients with inv dup (15) and UPD15 or 15q deletion/duplication [Robinson et al., 1993; Abeliovich et al., 1995; Brøndum-Nielsen et al., 1995]. It is difficult to correlate the two anomalies observed in patient 1 as the SMC is neither an inv dup(15) nor it contains the homologous 15q11-13 sequences. It could be that the instability of the pericentromeric chromosome 15 region and a mispairing during meiosis might have generated the two abnormalities without a direct causal relation. Although evidence for this hypothesis might be provided assessing a different parental origin, in most cases the small size of the SMC's does not allow this investigation.

Patient 2, with maternal UPD15, was shown to have a X-derived SMC. Also in this case we could not find a correlation between the two events. The most likely explanation is the occurrence of different meiotic errors because of the advanced maternal age at conception. Three cases of UPD in association with SMC's have been reported: one PWS and one AS patient with inv dup(15) and UPD15, respectively [Robinson et al., 1993], and a patient with transient diabetes with paternal UPD6 and a chromosome 6 derived SMC [Temple et al., 1995]. Based on the findings, James et al. [1995] speculated that individuals with SMC's might have an increased risk of being uniparentally disomic for the homologues from which the SMC is derived. Our data apparently contradict this expected association. Indeed our patient 1 with a chromosome 15-derived SMC does not have UPD15 (that accounts per se for about 30% of PWS cases) but rather the classical 15q11-13 deletion, while patient 2 has UPD15 and a X-derived SMC. These results should be considered in future studies aimed at searching for UPD of the chromosomes from which SMCs are derived.

The contribution of the marker chromosomes to the clinical phenotype in our patients is difficult to assess. A distamycin A/DAPI positive r(15) was found to be associated with a normal phenotype [Callen et al., 1992]. As the mar (15) observed in our patient 1 is the first reported of this kind, no information on its possible phenotypic effect is available. All typical manifestation of PWS except for hypopigmentation and characteristic face were present in our patient and no additional signs were apparent. Abnormal facial traits, developmental delay and seizures have been reported in two patients with a small ring (X) chromosome [Daniel et al., 1994; Silahataroglu et al., 1995]. However, no history of seizures was recorded in our case 2 but only cortical-subcortical irritation signs observed at 9 months and no longer present at age 5. Other differently sized mar (X) chromosomes have been described [Wolff et al., 1994], but the clinical signs present in some of the patients partially overlap with PWS features, making the comparison difficult.

Thus, two possible conclusions can be drawn from the available data: either the SMC's in the two PWS patients are almost entirely heterochromatic and without any phenotypic effect, or they are associated with a mild phenotype which is obscured by the PWS phenotype.

ACKNOWLEDGMENTS

The authors thank prof. G. Neri for critical reading of this manuscript and Dr. C. Romano for obtaining blood specimens. We also thank the families of the patients for their cooperation. This work was supported by a grant (5504/ICSS49.4RS92/699) from Ministero della Sanita' to Centro Auxologico Italiano for PWS and AS research.

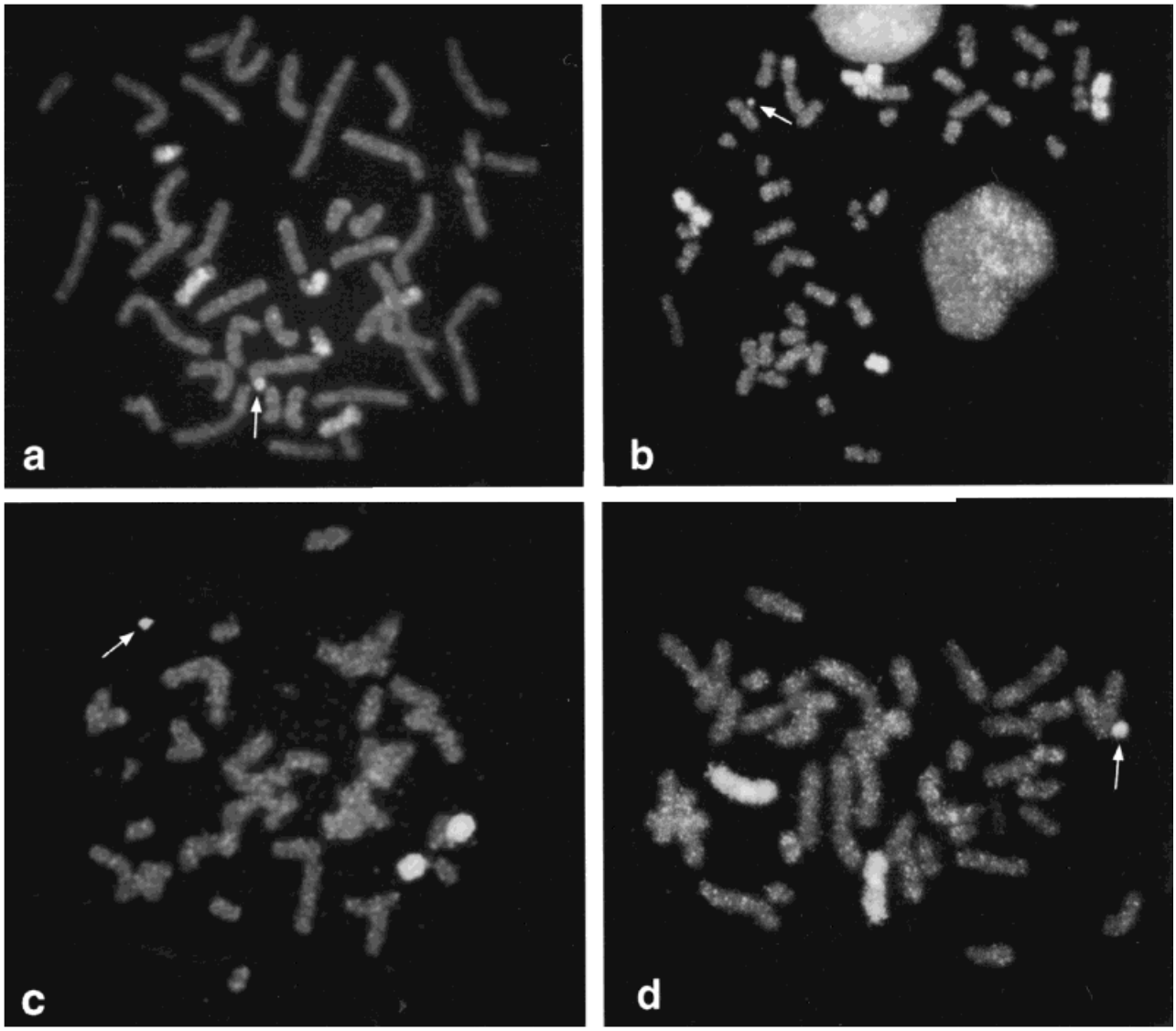

Fig. 5. CISS hybridization with mixed libraries for chromosomes 15, 21, and 22 to a metaphase from case 1 (**a**) and for chromosomes 3, 19, and X to a metaphase from case 2 (**b**). The SMCs (arrow) are painted in addition to the targeted chromosomes. After sequential CISS hybridization with the single libraries the marker (arrow) is shown to be positive with chromosome 15 (**c**) and X (**d**) painting probes in case 1 and in case 2, respectively.

REFERENCES

Abeliovich D, Dagan J, Werner M, Lerer I, Shapira Y, Meiner V (1995): Simultaneous formation of inv dup(15) and dup(15) in a girl with developmental delay: origin of the abnormal chromosomes. Eur J Hum Genet 3:49–55.

Blennow E, Bui TH, Kristoffersson U, Vujic M, Anneren G, Holmberg E, Nordenskjold M (1994): Swedish survey on extra structurallly abnormal chromosomes in 39105 consecutive prenatal diagnoses: Prevalence and characterization by fluorescence in situ hybridization. Prenat Diagn 14:1019–1028.

Blennow E, Brøndum-Nielsen K, Telenius H, Carter NP, Kristoffersson U, Holmberg E, Gillberg C, Nordenskjold M (1995): Fifty probands with extra structurally abnormal chromosomes characterized by fluorescence in situ hybridization. Am J Med Genet 55:85–94.

Brøndum-Nielsen K, Mikkelsen M (1995): A 10-year survey, 1980–1990, of prenatally diagnosed small supernumerary marker chromosomes, identified by FISH analysis: Outcome and follow-up of 14 cases diagnosed in a series of 12699 prenatal samples. Prenat Diagn 15:615–619.

Callen DF, Eyre H, Yip M-Y, Freemantle J, Haan EA (1992): Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. Am J Med Genet 43:709–715.

Cheng SD, Spinner NB, Zackai EH, Knoll JHM (1994): Cytogenetic and molecular characterization of inverted duplicated chromosomes 15 from eleven patients. Am J Med Genet 55:753–759.

Daniel A, Malafiej P, Preece K, Chia N, Nelson J, Smith M (1994): Identification of marker chromosomes in thirteen patients using FISH probing. Am J Med Genet 53:8–18.

Dittrich B, Robinson WP, Knowblanch H, Buiting K, Schmidt K, Gillessen-Kaesbach G, Horstemke B (1992): Molecular diagnosis of the Prader-Willi and Angelman syndromes by detection of parent-of-origin specific DNA methylation in 15q11-13. Hum Genet 90: 313–315.

Fleischer Michaelsen K, Lundsteen C, Hansen FJ (1979): Prader-Willi syndrome and chromosomal mosaicism 46,XY/47,XY,+ mar in two cases. Clin Genet 16:147–150.

Fujita H, Sakamoto Y, Hamamoto Y (1980): An extra idic(15p)(q11) chromosome in Prader-Willi syndrome. Hum Genet 55:409–411.

Holm W, Cassidy SB, Butler MG, Hanchett JM, Greenberg F, Whitman BY, Greenswag LR (1992): SB Cassidy (ed): "Diagnostic Criteria for Prader-Willi Syndrome." NATO ASI Series, Vol H61, "Prader-Willi Syndrome." Berlin, Heidelberg: Springer-Verlag.

James RS, Temple IK, Dennis NR, Crolla JA (1995): A search for uniparental disomy in carriers of supernumerary marker chromosomes. Eur J Hum Genet 3:21–26.

Ledbetter DH, Mascarello JT, Riccardi VM, Harper VD, Airhart SD, Strobel RJ (1982): Chromosome 15 abnormalities and the Prader-Willi syndrome: A follow-up report of 40 cases. Am J Med Genet 34: 278–285.

Mattei MG, Souiah N, Mattei JF (1984): Chromosome 15 anomalies and the Prader-Willi syndrome: Cytogenetic analysis. Hum Genet 66:313–334.

Robinson WP, Binkert F, Ginè R, Vazquez C, Muller W, Rosenkranz W, Schinzel A (1993): Clinical and molecular analysis of five inv dup(15) patients. Eur J Hum Genet 1:37–50.

Sachs ES, van Hemel JO, den Hollander JC, Jahoda MGJ (1987): Marker chromosomes in a series of 10,000 prenatal diagnoses. Cytogenetic and follow-up studies. Prenat Diagn 7:81–89.

Silahtaroglu AN, Hacihanefioglu S, Tarkan Y, Cenani A, Tumer Z (1995): A small supernumerary marker chromosome X identified by in situ hybridization. Clin Genet 47:270–273.

Spinner NB, Zackai E, Cheng SD, Knoll JHM (1995): Supernumerary inv dup(15) in a patient with Angelman syndrome and a deletion of 15q11-q13. Am J Med Genet 57:61–65.

Temple IK, James RS, Shields JPH, Crolla JA, Sitch FL, Betts JD, Jacobs PA (1995): Transient neonatal diabetes mellitus and paternal uniparental disomy of chromosome 6. Nat Genet 9:110–112.

Wisniewski LP, Witt ME, Ginsbert-Fellner F, Wilner J, Desnick RJ (1980): Prader-Willi syndrome and a bisatellited derivative of chromosome 15. Clin Genet 18:42–47.

Wolff DJ, Brown CJ, Schwartz S, Duncan AMV, Surti U, Willard HF (1994): Small marker X chromosomes lack the X inactivation center: implication for karyotype/phenotype correlations. Am J Med Genet 55:87–95.